Supplementary Protocol

One-step sequence- and ligation-independent cloning (SLIC): Rapid and versatile cloning method for functional genomics studies

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Procedures

1. Digest vector with restriction enzyme(s) overnight, and purify the linearized vector with a commercial PCR purification kit. Elute the DNA with elution buffer or 10 mM TrisCl, pH 8.0-8.5. Do not elute the DNA with water or TE. Measure the concentration of the vector.

2. Amplify your gene of interest by PCR using primers with ≥ 15 mer homology extension to the linearized vector end. We usually use 15 bp homology for single fragment cloning, and 20 bp homology for multiple fragment cloning. Purify the linearized vector with a commercial PCR purification kit. Elute the DNA with elution buffer or 10 mM TrisCl, pH 8.0-8.5. Do not elute the DNA with water or TE. Measure the concentration of the insert(s).

3. Mix the linearized vector and insert at a molar ratio of 1:2 in a 1.5 ml tube. An example is shown as follows. (Vector to insert molar ratio of 1:1 to 1:7 works well, but we usually use 1:2 for single fragment cloning, 1:2:2 for multiple fragments cloning. An example of 3 fragments cloning is shown below with vector: insert 1: insert 2 molar ratio is 1:2:2 as shown in Fig. 4A).

	Stock concentration	Volume added	Final concentration
Linearized vector (eg, 5 kb)	100 ng/μl	1 μl	10 ng/μl
Insert 1 (PCR product, eg, 1 kb)	40 ng/μl	1 μl	4 ng/μl
Insert 2 (PCR product, eg, 1 kb)	40 ng/μl	1 μl	4 ng/μl
10X BSA		1 μl	1X
10X NEB Buffer 2		1 μl	1X
H_2O		Up to 10 μl	

4. Add 0.2 μl of T4 DNA polymerase (3 U/μl, NEB) to the mixture and incubate at

room temperature for 2.5 min. We found that 0.5 μl (1.5 U) of T4 DNA polymerase gives the best result, but 0.2 μl gives more than sufficient number of colonies.

5. Put the reaction mixture on ice immediately to stop the reaction and incubate on ice for 10 min.

6. Thaw chemically competent E. coli cells on ice for ~10 min.

7. For single fragment cloning, gently mix the cells with 1-2 μl of the reaction mixture and incubate the cells on ice for 20 min. For multiple fragments cloning, mix the cells with 3-5 μl of the reactant.

8. Incubate the cells on ice for 20 min.

9. Heat shock the cells at 42 ^{o}C for 45 sec.

10. Incubate the cells on ice for 2 min.

11. Add 900 (or 950) μl of LB broth to 100 (or 50) μl of the cells and transfer the cells to 15 ml round-bottom tube.

12. Incubate the cells at 37^{o}C for 1 hr.

13. Plate the cells on agar plates containing suitable antibiotics (eg, 100 μg/ml ampicillin). We usually spread 10 to 20 μl of the cells onto an agar plate to get optimal number of colonies per plate for single fragment cloning, and 100 μl per plate for multiple fragments cloning. In case of 10-20 μl spreading, cells needs to be further diluted with 80~90 μl LB broth for suitable spreading.

14. Incubate the plates at 37^{o}C for 16 hr and analyze the colonies.